

Zelle single cell isolates

~~702~~
702

2/24 - done by Zelle 2/21/50.

A.	LAC	HAL X	MIL	G	H	I	J
47	+	+	±	34	++	++	++
48	+	++		37	+	+	+
52	+	++		38	-	+	+
55	+	++		39	+	+	+
56	+	++		51	+	+	+
57	+	++		82	+	+	+
58	+	++		105	++	++	++
59	+	++		106	++	++	++
61	+	++		061	-	(+)	(+)
62	+	++					
190	+	++					
91	+	++					
92	+	++					
93	+	++					
99	+	++					
103	+	++					
121	+	++					
169	+	++					
170	+	++					
189	+	++					
201	+	++					
202	+	++					
246	+	++					
491	+	++					
492	+	++					
10	+	++					
13	+	++					
16	+	++					
18	+	++					
19	+	++					
20	+	++					
29	+	++					
30	+	++					
35	+	++					
36	+	++					
48	+	++					
54	+	++					
95	+	++					
96	+	++					
17	+	++					
20	+	++					
21	+	++					
22	+	++					
23	+	++					
24	+	++					
26	+	++					
31	+	++					
32	+	++					
33	+	++					

Hal X

++ # # #

All ± unless indicated

Restreaks A169, A189, C30,
E116, E121, H17 m
E140 Hal

C30, m E143 Xgl.

All still deploid!

March 6, 1950.

- A. Diphenyl iodonium chloride 3.7 mg/ml in D(0) 10 ml + 1 ml H226
 at 37°C. 10 minutes + plate out. dilute as treatment tube.
- B. Acetone acetylide per 692 C. dil. from treatment tube.
- C. Control. 10^{-7}
- D. Pt. $\phi_2 \text{ICl}$ 28 mg in 10 ml D(0). Add 1 ml H226. incubate 30 mins
 at 37°. Plate out.

Results.

A: Plates were ~~not~~ discarded before counted, but killing was]
 < 50%. Some haploidizing effect? } See
 C. ?? Ca 90% diploid 10^9 . [Discarded]. } 70γ

B: slow growth: hold.

48 hours: Dilution $\frac{1}{2}$. 10 Lac- 0 Lac+

Dilution $\frac{1}{1}$ 82 Lac- 1? Lac+ Stuck out: Lac+

Hold plates further:

at 72 hours, no further + appeared; no residual + in centers.

Note: Very low proportion of "residual" diploids with
 high doses of Ac_2O .

March 8, 1950.

Use once washed H226 (3/5/50) in D(0) in this series. 37°

- A. $\text{O}_2\text{I}^+ \text{Ce}^-$ 25 mg / 10ml D(0) + 1ml H226. 30 min.
- B. IAcNH_2 (Doherty - Brown sol'n : must contain free I_2) 50 mg in 1ml + 10ml D(0) + 1ml H226. 25 minutes.
- ✓ C. Benzoyl chloride : 2% alcoholic. Add .1ml to 10ml D(0); 1ml CaCO_3 , + 1ml H226 10 mins.
- ✓ D. Dimethyl sulfate 10% alcoholic Add .1ml to 10ml D(0) + 1ml H226 + 1ml CaCO_3 10% 15 min.
- ✓ E. Phenyl isocyanate : 2% alcoholic. Add .1ml to 10ml D(0) + 1ml H226. 10 mins.
- ✓ F. Ethyl carbamate (urethane). 2.5 ml 20% solution + 12° to 31° (ca 2 hours, 5%). 1ml H226 + 6.5ml D(0)

Results:

A 5 ca 200 \approx 85% bacv. Killing too meager to be decisive.

B Stains at every dilution. Repeat at lower concentrations! ✓

C 6 ca 100 mostly 2n - Killing too meager

D 1 ca 100 all bac - Later some delayed Lacy.

E 6 ca 100 mostly 2n - Killing too meager.

F 2 ca 100 90% Lacy.

Use larger intervals with Benzoyl Chloride; Phenyl Isocyanate

A. H226 3/5 washed 1ml in D(0) 7ml + Propylene Oxide 2ml 10%
 37° $\frac{345}{345} \rightarrow .830$! STERILE

B. do. 50mg Ninhydrin in 4.5ml D(0) .5ml H226 37° . 50 mins.

C. IAcNH_2 1% $1:10 \left\{ \begin{array}{l} \text{D(0)} \\ \text{+ 1ml H226 } 37^{\circ} \end{array} \right.$ ^{Excess inhibitory}
 D. " $0.1:10 \left\{ \begin{array}{l} \text{D(0)} \\ \text{+ 1ml H226 } 37^{\circ} \end{array} \right.$ ^{No killing} 10mins.

A. Use shorter treatment; more dilut. $P_2 < 0$

B. Not haploidizing $\theta 2:$ ca. 100; 2y.

C. $2:4$ ~~goats~~ is 5 day, 1 day - ; C 1: several hundred; several hundred bacv

D. 6 ca 100 bacv. ^{centrifugally} ^{purifying bac -}.

Chemicals in H226

706

March 10, 1950.

- A. Benzoyl Chloride } see ~~704~~
 B. Phenyl carbonate } ~~705~~ C + E. ~~100 P.M.~~
 • 2ml 2% solution + 1 ml H226 3/9/50 washed + 10 ml D(0)
 + 1 ml 10% CaCO_3 for decoe.
- ~~1 hour~~
- C. K_3FeCN_6 M/100 1 ml + 10 ml D(0) + 1 ml H226 10 m.
 D Lumine Sulfate 0.1% 1 ml + " " 50S
 E Iodine M/20 1 ml + 10 ml ... 10 m.
 F " 0.1 ml 10 m.
 G H_2O_2 final concentration 0.03% 10 m.
 H H_2O_2 added to Penicillin to 3%. Ascorbate 5% - 8%.
 Add 1/10 to D(0) and add 1 ml H226 10m. = 6.
 I CH_3COOC 1/10 in EtOH. .01 /10 ml D(0) + 1 ml H226 H226.

A. A4 Ca 150 ~~at~~ 90%+ diploidREPEAT!

B Sterile

C6 : ca 100 diploid 90%. No killing!D6 ca 100 haploid ca 90% Inadequate killing

E 1-6 Sterile

F Sterile

G5: 30Lac - 17 Lacv.

H6: 6Lac - 11 Lacv

H5: 65 Lac - 36 Lacv. Kaptonized? ✓

I6 ca 100 diploid 90%.

(over)

Native peroxide and peroxide-treated both appear to
be equally active as ~~not~~ bleaching agents.

700-3 x y10

207

"Diploid ???"

March 10, 1950.

700-3 x y10 as EMS Lac.

Ca 600 phototographs : all her +

Chemicals on H₂26

~~708~~
708

March 13, 1950.

3/9

C. H₂26 3/8 1 ml + D(O) 10 ml + CaCO₃ 10% 1 ml + CH₃COCl 0.1 ml
Immediate hydrolysis observed from CO₂ evolution. Keep cold
in space with tube i same addition, but let stand 4 hours. Before
adding bacteria.

N.K.

D²

N.K.

E Benzoyl Chloride + 10% CaCO₃. + 1 ml H₂26 Shakes at Room
temperature. 1 min. N.K.
0.1 ml

F. Propylene oxide to 1%. In D(O) - 1 ml H₂26 10 min

G. Caffeine citrate to 0.3% N.K.

H. K₂S₂O₈ to 1/200 N.K.

I. K₃Fe(CN)₆ to 1/100 N.K.

J. I₂ to 1/20,000.

Dil. Lacv Lac-

CH ₃ COCl C	6	25	27	Effective (?)	CH ₃ COCl
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Hydrocl. D	6	101	14	CH ₃ COOH + HCl
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PhCOCl E	6	49	9	Inconclusive	N.K.
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P ₂ O ⁵ F	6	98	17	Inconclusive	N.K.
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Caffeine G	6	99	14	Inconclusive	N.K.
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Perchlorate H	6	117	7	"	"
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Ferricyanide I		149	10	"	"
Toxidine J { 3	4	41	8	Does not haploidize	: 10 ⁻³ survivors!
			7		
			4		

March 14, 1950

St(X) Benzoyl chloride (+ D(+)·CaCO₃) Shake at RT 1100 AM to 1PM
H₂26 3/4 STERILE

L. CH₃CHO to 0.5% H₂26 3/4 unev. 20 min. 37 °K

St(M) φCMO .1ml 1/10 " " 4 hours ~~to 30 min.~~ - Shake at RT.
(=.06 ml) STERILE

St(N) HNO₂. Add ^{to} 10ml 1/10 KNO₂ ~~to~~ 1mM AcOH. Make up to 1/50
in pH 4 citric - KP buffer. Add 1ml H₂26 w. 3/4 water for
effect of HNO₂. g.o. STERILE } 30 minutes.

O. No KNO₂ - AcOH; buffer only. } ox.

P. H₂O₂ 0.03% Dark 5ml H₂26 3/4 wash / 10. Sediment and resuspend

G " " Light 30 min.

R " " Dark control (cool in dark while g is illumination.)

P15: K, M, N - all sterile.

L6: Lacv 81 Lac- 51 Possible effect.

O5: 9 6
4: 91 78 Possible effect!

P6 (no rotation
for extra
concentration) 158 81 } Inadequate

G6 146 47 } Killing
R6 106 47 }

March 15, 1950

- S. ~~MeCHO~~ to 1% H₂2G 3/14 w. 30 m. 37
 T Propylene oxide to 2% " " 30 m. 37
 U. Ethylene oxide to 1% " " 30 m. 37
 W pH 4 citrate buffer { H₂2G, washed in water.
 X Control, water. } 8²⁵ - 15 mins.

pH 4	W 4:	Lacv	Lac-	
	S:	13 3	27 4	too heavy for accurate score, but - clearly > Lacv.
X	6	75	8	small but countable
(control)	6	76	7	
	6	41	3	The increase in proportion of haploids is beyond question.
<chem>CH2=CH-O-</chem>	U 6	38	44	Definite haploidization. Many are 0 types.
<chem>CH2=CH-C(=O)CH3</chem>	T 6	68	35	" "
Acetaldehyde S 6		25	9	Uncertain?
	SS			- Some increase in proportion of haploids??

P16. Y H₂2G (H₂O) in Acetate-Na-buffer, pH 4, 4/10. 15 mins.

36 hr. rdg. Lacv Lac-

4	12	19	{ indistinct.
3	138	83	augmentation of
			haploids.

= (over) ≈

2. Potassium hydrogen phthalate buffer
pH 4.0 19/20.

15 minis 37°. H226 (H₂O) 1:10

Lac+ Lac-
23: 108 11

Despite 3 decades of killing, no alteration of hybrid-dyloid ratio is found. The effects of previous sections may be ascribed to acetic and citric acids respectively

($\rho K_{(1)}$) = 4.76; 3.06

But ρK (phthalic) is 2.89, 5.41, so at pH 4 there should be as much free phthalic as citric!

Photorecovery of peroxide-treated
E. coli

709

March 13, 1950.

H_2O_2 to .05% in 1(0) ~~6~~ ml + 4ml H226 3/5.

Incubate 10 mins., sediment 10 mins. Divide washed suspensions
into ^{equal} aliquots, ^{dil. 1:1 v/v}, one to be exposed to visible light 30 minutes.
305 - 405. Control left at room temperature.

A (light) sterile

B (dark) B1 3Lac - 1 Lac

Dose too high!

Partial segregation of H168

711

3/11/50.

Grow H168 from single E14S lac colony in D(Lac). Dose 1:1000 in Y2 and grow, aer., to saturation. Plate out on E14S Lac, Xyl, Mtl. 10^{-6} : ca 500 / plate. Pick - colonies and re-purify.

EHB \rightarrow Lac ~~Mtl~~ Xyl

- A. Lac
- B. Xyl
- C. Mtl

A. 9 ---

B. 24 23 Lac+ Mtl-Xyl-
1 Lac- " "

C. 16: all Xyl-Mtl- 3 Lac- 13 Lac+

No lac₊ ∴ No partial segregation.

No partial segregants found in H-168

March 15, 1950.

Growth from 1:1000 in Y2. Plated out on E14S 10^{-5} .

A. Lac. Considerable Lac- (ca. 30% of count of 3-500/plate).
1 pick probable pure Lac- and brushed on E14B Mal, Xyl, MH, Lac for discrimination of Lac- ... v.

All are, indeed, pure Lac-. Scoring of v coagulant + is possibly uncertain.

⁶⁹⁹ 1 prob. Lac- MH v { noted. Recover and retest. ^{Lac} - Xyl v Mal v MH v
²⁰¹ 1 prob Lac- Mal v { noted. Hold for appearance later. v v MH v.

~~All test~~ of others, 96 Lac- Mal + MH + Xyl +. 2 ----

of an additional 100, no Lac- MH, Mal, or Xyl v noted, subject to deficiencies of the brushing technique.

B) MH. <10% of prototrophes possible MH-. of 20 picked, 6 are MH-

c) Mal 4 Mal -

D) Xyl 10 X -

B: 6 tests 5 are Lac- Mal + Xyl + 1 is Xyl slow = #3 (distinct from + or -)
 Mal Xyl MH Lac
 C: 1 - v v v
 2 - + + -
 3 - - + -
 4 - - - -

D: 10: all Lac- 5 MH + 9 Mal + all Xyl -
 5 MH - 1 Mal -

No Partial segregants except C1
do not keep.

H168 - Mal-reverse
for hemizygosity test

713

March 15 ff 1950.

Reisol H168; grow in O(lac); culture ca 85% Lac^v.

A. streak on EM5 Mal to select reversions.

Papillae picked en masse 48-72 hours. Streak on EM5 Mal to purify. N21 Pick "single" + colonies and streak

B. As above

C. Dose ^{hairy} 3 tubes of O(Mal) anaerobic. Growth after 48 hours. Streak out to purify.

D. Single Mal- colonies from EM5 Mal as same for reversions.

A: 3 Mal+ prototrophs. 2 are Mal++ Lac^v

1 is Mal++ Lac +.

∴ 2 additional tests for hemizygosity of Mal in H168.

C: 3 Mal+ prototrophs all Mal++ Lac^v.

" "

B: 8 Mal+ " " " "

" "

Acid effects on diploid coli

714

March 21, 1950.

? See 716

A. Acetate pH 4 M/10 H₂O₂ 2/21 15 minutes.

B. " pH 4 M/100

C. Phthalate pH 4 M/20

D. " " M/100

E Control

This culture apparently
contains some bac.
Streak out as 714-A

	bacv	bac-	Lact+	
A. 5	620	34	20	??
6	2	5	2	

very little killing !!

B 6	115	3	6
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C 3	93	3	7
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D 6	29	5	4
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E 7	121	2	8
"	133	5	7

Utilization of neolactose

715

March 22, 1950

A	58-161	Mal	1cc cells from 4/10 case. D(O) medium.
B	"	Lac	aerated overnight; twice washed
C	W1301	Mal	Bicarib buffer 7/20. .2ml 2% substrate
D	"	Lac	

Flesh culture substr.

	^{n Hars.}		
1	10B	B	-
2	5B	B	Lac
3	3A	B	Neolac
4	6A	D	-
5	2B	D	Lac
6	4B	D.	Neolac

7/22/50

ThB	1	2	3	4	5	6	C ¹⁶
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230

82+	05+	42	51	29	32+	2+	
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235

85	05	40	49	28	32	1	
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240

90	83	8+	42+	51+	29	32+	1+
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245

86	-1	32	40	18	21	-6	
----	----	----	----	----	----	----	--

255

85	+1	35+	42	21	23	-5	6
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305

81	-4	36	42	19	22+	-5	
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410

81	-4	52+	47	16	22	-10	
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No fermentation at all!!

H226 segregants for outcrossing

March 22, 1950.

Pick single lac^v, from 714E7, streak on EMBA Mal.
colonies

All were pure ~~to~~ Mal-! Very likely suspension of H168 was used in this experiment and in 714. This would account for low killing as H168 is suspended in buffer.

Appearance of lac^v is not unlike H168!

Repeat from H226.

10 Mal+ and 10 Mal- (conjugate) isolated and mutators tested

		+	-		lac	Mal	-	Xgl	Mal
W1305*	1	MTL	"	TLB,	W130Y	-	- +	-	- +
	2	TLB,	"	TLB,		-	- +	-	- +
	3	TLB,	"	TL		-	- +	-	- +
	4	TLB,	"	TLB,		-	- +	-	- -
	5	TLB,	"	TLB,		-	- +	-	- +
	6	TLB,	"	TLB,		-	- +	-	- +
	7	TB,	"	TB,		-	- +	-	- +
	8	TLB,	"	TLB,		-	- +	-	- -
W1303*	9	M	"	TLB,		^{blue} early	- +	-	- +
	10	TLB,	"	TLB,		-	- +	-	- +

check fermentative reactions.

∴ #9 can be presumed to be the recovered B-M- parented W67 type.

Cross with #11, and with W1177 lac+.

Hold W130Y for lac+ reversions for crossing.

Alma P. W.
May 2, 1950

5-224 = segregant

5-223 = 2n : nucleate.

5-86 Lac+Mal+

5-85 2n

20:

Partial segregation:

8 Lac+ and 8 Mal- prototrophs from H206 tested
each was Lac+ Mal-

Studies on single cell segregants
(14226)

717

Recovered from vials sent by MR Zelle and stored in refrigerator.

Series 2/5

A15 * inviable A33 also inviable!

D- some inviable on EMB agar. D15 and D20 are Lac-Mal-Xyl-Mtl -
others inviable!

E 215 ✓
216 ✓
~~221~~ x?
222 ✓

F 103 ✓
104 x ✓
105 ✓
106 ✓
107 ✓
108 x ✓
109 x ✓
110 x ✓

* Recovered
from E14 Blue
agar!

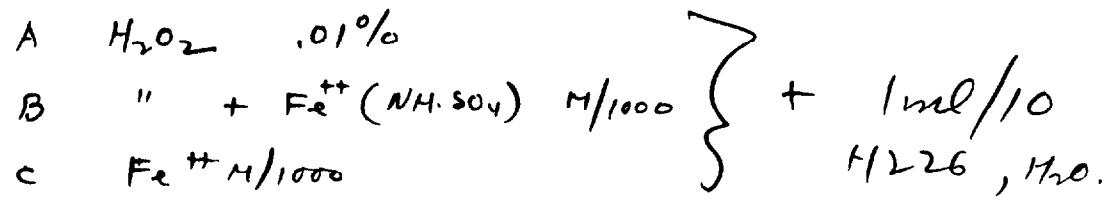
Sub-leptoids:

G3 G13 inviable E 219 viable OK
G9 01K 220 n.v.
G24 01K 52 n.v.
108 n.v.

$[HO]$ radicals in H₂O₂
"Fenton's" Reagent.

718

March 28, 1950



Lar+ Lar-

A6 37 22

B6 23 27

C6 112 10

Ferrous by itself has no effect. No very marked potentiation of H₂O₂ observed. Should be repeated under slightly more drastic conditions.

March 31, 1950.

A. Benzoyl anhydride 0.4% (2/100 of 2% alc.) [sat'd. I.
in D(O) H226 3/4/50]

yr5 -

B. Benzoyl peroxide 0.4% "

C. Acetate buffer 4/10 pH 4

D. " " 4/50 pH 4

E. Phthalate " 4/20 pH 4.

F. Control 10^{-7} :

F 10^{-7}	taev	lac-
9	5	
10		
16	4	
	5	
65	14	

C 3: 8 101 ←

D 5: 5 12

D "4" 1 18

D 3 10^{2+} 178 548

ab6
862
213101-

061

E 2 5 10

E 1 131 70

} any effect?

April 1, 1950.

A $W67 \times W945$ 1/8 lac+, 12 plates, ca 100 each. (#18)
 B $W67 \times W950$ 1/8 " 14 plates

For recovery of presumptive diploids, reisolate (A - single col.) and (B - thimble streaks) from EMS lac and streak on EMStac EMStac Lac, Mal, Thal.

		lac+	B lac	B Mal	B Thal		
A18. A (from EMBO)	B		-, v v, -	+,-? +	++ +,-, v?		Reisolate from Stac.
B 1 A		lac-				Xgl Mal Mal	Mal Stac
2 B			n.g. on EMStac				
3 A B			v v v v v v	- =	v v - v + - ++		Mal - !
4 A B				= + - + ++	= - ++		
5 A B			+ (crossed out) + (crossed out)	- - - +	= = = -		
6 A B			v v v v	- + + +	= = ++ =		
7 A B			v v x	- + - +	- - - -		
8 A B			v v v v	- - - -	v +? = = v +? = =		
A 18			v	-	+ + v		

Keep A18, B3, ~~B5, B6~~; B6, B7, B8 when reisolated!

H	Lac	Mal	Xgl	Mal	Stac	Mal
233 A18	v	x - x +?	v			
234 B3	v	v	v		+	
235 B6	v	x - -	-		+	grows poorly
236 B7	v	x - -	-		-	
237 B8	v	x - v	v?		-	Use for reversion study

Effect of pH on segregation of H226

721

April 2, 1950.

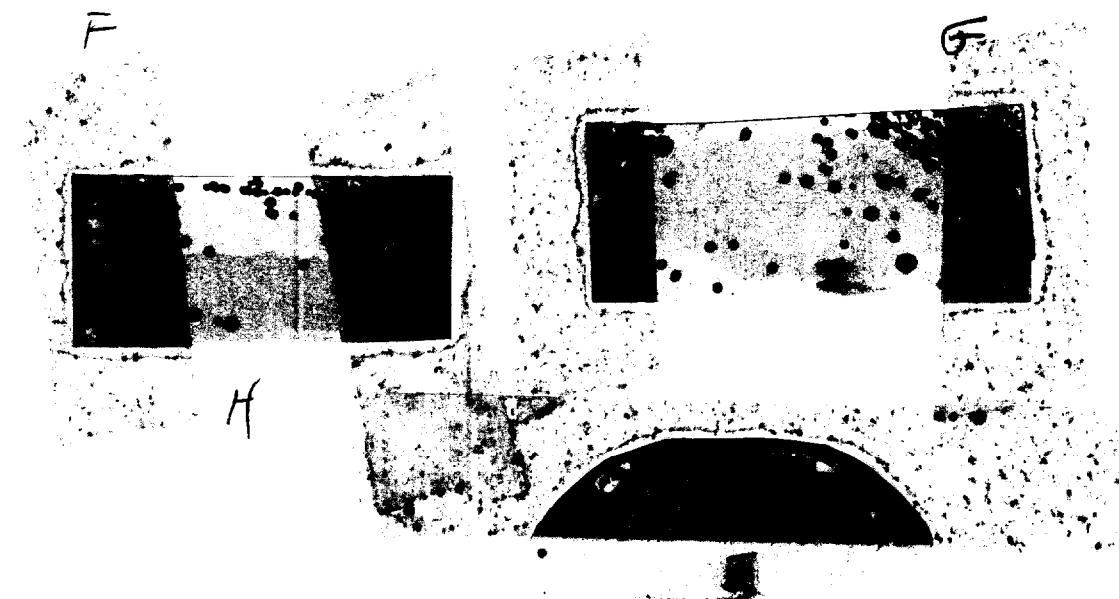
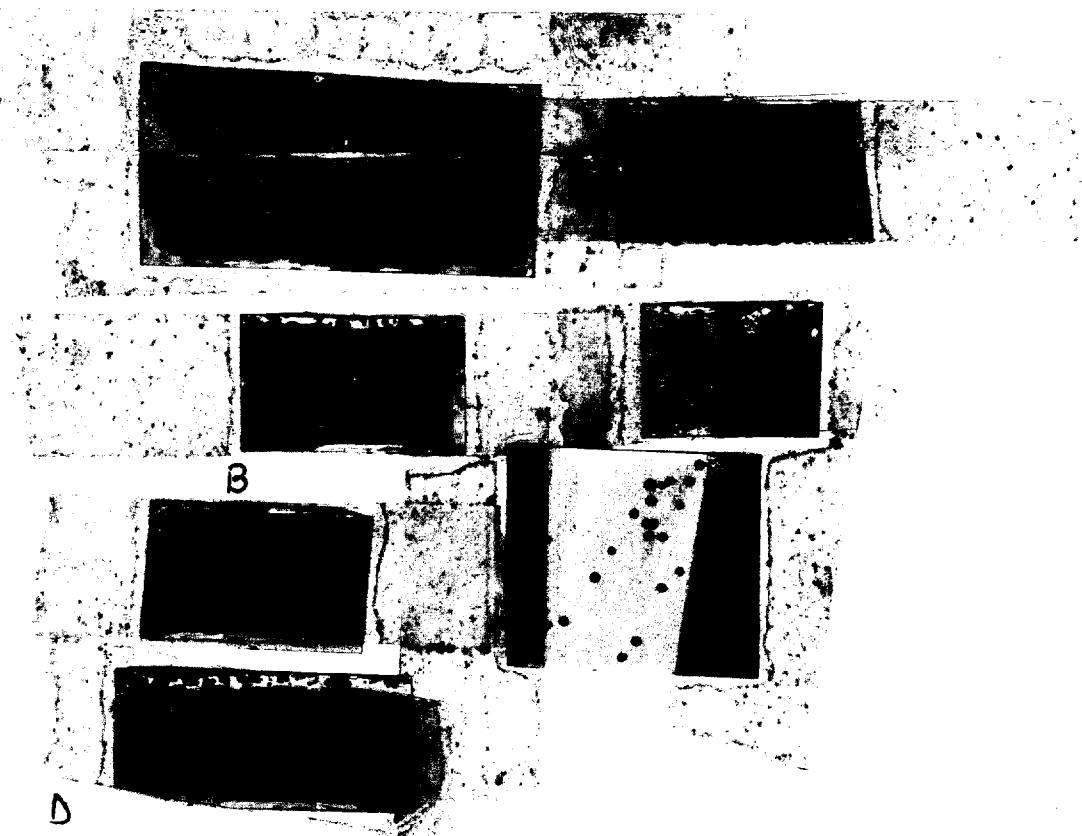
Prepare D(0) + 1% NZcase. Measurement of pH. Adjust to various lower pH's.

Medium:		24 hr.	48 hr. gr. % <u>se</u>
A D(0) + 1% NZcase		+	+++ 70
B " " + .1% glucose	++	+++ 50-60	
C " " + 1% glucose	+++	++++ <50	
D D(0) + 1% NZcase to pH 5.9 with AcOH		+	20
E " " " 5.0	-	long time	
F D(0) + .1% glucose		++ >80	
G D(0) + .1% lactose		++ 785	
H D(0) + 1% glucose		+++ 60	
I D(0) pH 6 0.1% glucose		+ 65	
J. D(0) pH 4.9 0.1% glucose.		± all -	

Streak out
on EM13
2cc at 2th.

Results indicative but should use smaller inoculum.

727



Outcrosses of H226 segregants

H22

April 2, 1950.

"F" W1304 x ~~W1304~~ 58-161 / EMS lac.

72 lac+ picked and streaked on EMB lac. None were lac_v.

~~Most - were lac+~~, possibly + > -.

Test on EMS Mal for Mal segregation:

	Mal+	Mal-
lac+	11	62

	Mal+	Mal-
lac-	10	58

A W1303 x W1304 13 plates EMS lac ca 200/plate = 2600

~~W1303 x W1304~~ 5 + colonies at 80 hours. hold plates for delayed test.

Pick these and streak on EMS lac EMB lac EMB 17al.

6 plates EMS Mal - dilute suspensions: yield lower
9 Mal- 2 Mal+ streak + as 6,7.

Both pure Mal lac-.

Lac	Mal	Rein. lac	Mal	Xyl	Mal	H
1	v	v	v	v	v	240a
2	v	v	v	v	v	240b
3	-v	-,+ v?	-v	-	v	241
4	v	v	v	v	v	240c
5	v	v	v	v	v	240d

Note the prevalence of Mal_v here

H241 should be tested for hemi/homo - zygosity of Mal.

44 colonies picked from A as darker after 3 days. Test all to look for faded lac v. None were after 4 days incubation.

April 10 ff. 1950.

E. W1303 x W945. (?) Does bal become heterozygous?)

P16: ~~10~~ 10 plates EMS lac. ca 200 scoreable prototrophs per plate.

8 possible lac+ or lac-. Pick and streak on Lac-EMS, Lac-EMB, Mal-, Gal-EMB.

6 = Lac_v. # 2 = Lac+ others Lac-. Results as 722-E1
Mal- Mal-

of 10 addnl plates (ca 1500...), 4 "+" picked for testing. 2-, 1+ lv.
E2
E2 is lac_v Mal_v clonal isolates

G. W1304 x W478. ca 1/3 Lac+. Pick 90 lac+ and streak on EMB lac.

5 possible lac_v. Repick to EMB lac; Mal EMS lac.

	lac	Mal
1	✓	-
2	✓	-
3	++	-?
4	✓	+
5	++	-? v?

E1 lac_v (weak) Gal-Mal-
E2 lac_v Gal_v Mal_v Xyl_v Mtl_v

Note: ~~no~~ Mal_v.

After additional 24 hours, a number of "brown" lac+ picked from EMS.

of 21 isolations, 16 are apparent lac_v. Repick these as above.

1	+	-
2	+	-
3	+	-
4	✓	-
5	✓	-
6	✓	+,- v?
7	✓	-
8	+,- v?	+
9	✓	-
10	✓	-
11	✓	-
12	✓	-
13	+,- v?	v?
14	✓	-
15	+	+

Mal_v?

No definite Mal_v but
Reacts.

April 20, 1950.

H. W1311 x W67 ("A") { on TMS Lac
 I W1311 x W1304 ("B") → no yield ^{Note 1/5/51} both are TIB.

H 100 lac+ picked and streaked as TIB lac

Hold possible v in refrigerator.

Pick purified lac⁺'s to DN2 Glu for spot test of constitutive lactase.

28 lac+ 26 mpg+ 2 possible mpg- (11, 18)

Replate these on ~~DN2~~ Glu for rechecks.

Re streak -1 for ~~at~~ photophase constitutive lac+.

12 lac slow 1 possible

Hemizygosity tests: lac - partial seg.

April 3, 1950.

Extracted from 699 - .

Type I (lac - Malv) : on EMS lac, may eventually assume + appearance.
Select "papillae" from D(0) agar - lactose.

699-1 No papillae found Colonies remain very small.

699-2. 2 papillae: #1 gave - and lac_v colonies on EMB lac
2 gave only lac - ! streaks.

699-20. 8 papillae: #1-7 lac_v. #8 lac - .
more appeared later!

But all of these cultures are lac - on EMS lactose !!
see below - eventually give + center colonies.

C 4/3: Pick single prototrophs from EMS lac A1.

D " " lac_v colonies from EMB lac A1

E From EMB lac B1-7, 1 each.

But after 3 days, colonies with dark centers appear on EMS lac,
probably representing "lac +". This appearance develops very slowly.

723C #1 and #3 are weak lac_v on EMB lac and show comparable
appearance on EMB lac. Restreak #1 on EMS lac. Transfer to
D(lac) slant as 723 A1 -: ✓ mostly weak lac_v.

723D More or less typical lac + on EMS; lac_v on EMB. Streaks out
to compare with 723 A1. A1 and D1 both give weak lac + on EMB

F 4 new colonies from 699-20 on D(lac), to EMS lac
after 72 hours, lac + centres.

Lac hemizygosity tests

723g.

April 7, 1950.

723: A + C compound:

Both are Lac - after 24 hours; but give lac^v mosaics apparent
in 48 hours on TMB Lac. On EMS Lac, colonies taken at 48-72 hours.
This holds for all of this series! How many lac^v may be missed?
Or, are these not true reversions?

See 722: no comparable lac^v failed isolated from ~~W1303~~ W1303 x W1504

Hemizygosity tests
Mal - partial sus.

724

4/2/50 ff.

From 699:

A.

699-11:

2 papillae : lacv Malv Save single MalEMS + colony from each as
724A1, A2 OK ✓ Cf. 699-11R1.

an additional 2 papillae to EMS Mal. 1 gave Mal+; the other only papilla
Resist. from second. Check'd as A3. (2d) - Festuca a, EMS Mal v/c check
as A4: 15 Mal- X- ∴ ++/- i.e. "E18"
Malv Lacv ✓

D

Grew 699-11 on D(Lac) aer., overnight. Spread on several EMS Mal and
D(Mal) plates to obtain additional Mal+ reversions. N10 About 50 cols on
7 plates. 30 picked and streaked out on EMS Mal. - Reprod. single colonies
and streak on TMB Mal, Kesson ^{D Lac} ~~EMS~~ Mal; TMB Lac -

#20 = Mal+ ^{Lac-} ~~l~~

Others are all lacv Malv.

#26 = Mal+ Lac?

each was Lac-

Hold on D Lac plate

B

699-9. 3 papillae from EMS Mal to EMS Mal for purification. Resist.
pure+, check, and to slants as 1-3 1/6

#2 lacv Malv Kupas
#1, 3 lac-Mal+ 724B1
reject others

C

699-12. 2 Mal+, both give Malv. 724C1, 2 purified and
recheck. To slants 1/6 lacv Malv ✓

Linkage phases of 699-11 14+ meusens. 724a

4/8/50

Streak out A0-A4 on EMB Mal.

P7. Restreak Mal_v. Pick isolated Mal_v - on the same plates and brush on EMB Mal.

	M _{v+}	Malt+	M _{v-}	M _{v+}	Malt-	M _{v-}	
A0	0	6		17	2		+/-/+
A1	1	5		5	1(v?)		+/-/+
A2	1	2		5	1(v?)		+/-/+
A3	0	0		0	1		+/-/- ??

P8 8 Mal_v from each of above streaked out on EMB Mal to obtain distinct segregants. Pick app. pure tankel - from each quadrant to ~~#~~ Xyl EMB. me + one -

A0.	Malt+	Mal-	A1	M+	M-	A2.	M+	M-	Each of these
1	8X-	15X+		X-	X+		-	+	
2	1X-	6X- 1X+		X-	X+		-	+	
3	5X-	5X+		X-	X+		-	+	
4	2X-	5X+		X-	X+		-	+	
5	3X-	6X+		X-	X+		-	+	
6	3X-	6X+		X-	X+		-	+	
7	8X-	8X+		X-	X+		-	+	
8	8X-	6X+		X-	X+		-	+	

A3.	M _{v+}	M _{v-}
1	4+	4-
2	3+ 1-	4-
3		4-
4	5+	4-
5	2+ 2-	4-
6	4-	4-
7	3+	4-
8	8-	1-

This is very likely in
the ++/-- phase.

Are the segregations actually
complementary?

For 724D: — into ~~#~~ Pennesay
plate out on EMB Mal for segregants to test linkage phases.

Lineage phases of 699-11 reversions

7246

April 12 ff. 1950.

A0 trans
 A1 trans
 A2 trans
 A3 cis
 A4 cis
 D cis

} See 724a.

	Mal +		Mal -	
	X+	X-	X+	X-
A4	10	0	0	1
D1	10	0	0	0
D2	10	0	1	3
D3	10	0	0	0
D4	10	0	0	4
D5	10	0	2	1
D6	10	0	0	4
D7	10	0	0	1
D8	0	10	1	0
D9	9	1	0	2
D10	10	0	0	3
D11	10	0	0	2
D12	10	0	0	2

++/-
 Count up 1

Recheck.

All but D8 had a preponderance of + segregants

D1-12 appear all to be in the cis phase ++/-. However, since they were recovered from a single plating they might represent recurrences of the same mutation and should be counted as but a single reversion, v12., D.

Reinitiate the experiment by starting cultures from separate single colonies.

E ~~#22~~ new, independent reversions on Mal, from EMS Mal from a single bac+ (E15) colony. There were bac-, Mal+ pure (segregated!) pure bacu Malv (#10, 13, 15)(21, 22) (Recheck 22: maybe bac+ Mal+)

(See over)

F

Type reads as:

	Mol +	Mol -	Type
E 10	10 - 0 +	10 + 0 -	trans
13	12 + 0 -	10 - 0 +	cis
15	10 + 0 -	10 - 0 +	cis.
21	13 + 7 -	18 - 0 +	cis
22	20 + 2 -	18 - 0 +	cis

F 1	10 - 0 +	10 + 0 -	trans (Note: Lac-)
2	10 - 0 +	5 + 3 -	trans
3	6 - 0 +	2 + 1 -	trans
4	11 + 0 -	11 - 0 +	cis
5	11 - 0 +	7 + 2 -	trans

~~see 724c (do not accumulate)~~

G. From dilute plating of H238 (D lac) at EMS 14 al.

1-4 OK.
{ 2-3 OK.

Cumulative score:

TRANS IIII IIII I

213 IIIIIII IIII II

724 protocols

F8 724 F8 Malv Lacv

"9" Mal+ Lac- segregated.

Definitive

G5 Mal+ Lac-

6 Mal+ Lac-

7 Mal+ Lac-

8 Mal+ Lac-

G5 Malv Lacv

10 Mal+ Lac-

	Mal+	Mal-
F6	8+ 2 -	8 -
F7	9+ 0 -	10 -
F8	10 -	10 +

cis
cis
trans

G1	10+ 0 -	7 -
G2	10+ 0 -	9 -
G3	10+ 0 -	3 -
G4	9+ 1 -	6 -
G5	10 +	8 -

} 5 cis!

cumulative score:
9T 11C

G6	a	Lacv Malv +	10 M- X+	4 M+ X- 3 M+ X+	TRANS
G7	b	Lacv Malv +	8 M- X+	2 M- X- 10 M+ X-	TRANS
G8	c	Lacv Malv +	10 M+ X+	9 M- ? X+	??
	d	Lac- Mal+			

G8 was almost completely M-. Repeat.

11T 11C

5/23 G8 is pure Lac+ Lacv Malv (partial segregant?)

Associated Mal- should be saved to determine whether there is any correlation of the mutation with partial segregation.

H like G. "1-11" purified. 1,2,4 were Lacv Malv; others were Lac- Mal+.

	M+	M-
1	7 X+ 3 -	10 -
2	9 X+ 1 -	10 -
4	9 + 1 -	8 -

#3 had Lacv minor component. Calculate score
Lac+ 11S. Eliminate designations 3-11.
Not Lacv; Lac- Mal+.

Rest of H. is all Lac -

May 10, 1950.

Apparently, a partial segregation occurred after mutation from ~~Mal-~~ to Mal^+ , resulting via lac- \rightarrow tools. The Lac+ and - components present in the finally isolated Mal^+ prototroph (a kuehne on EMS Mal) were separated. Each was Mal_V . The Lac+ was Lac_V . The Lac+ component must be ancestral; score it for Mal-Xyl linkage phase with remainder of series. Reg lac- as a partial segregant. $F1+ : 7\text{Mal}- : \text{Xyl}+ : 8\text{Mal}+ : \text{Xyl}-$ also trans

$F1-$ apparently gives somewhat mosaic colonies on EMB Lac after 48 hours, resembling the "lac+" colonies of H239. (Lac+ trans 699-20 loc-)

Repick single colonies from EMS Lac (4 colonies)

24h. Each is Lac - Mal_V .

60-72 hours. On Lac, definite mosaicism with dark centers shown by most colonies. On EMS Lac, some colonies are much darker than others. These.

The "dark" type gives colonies on EMB lac mosaic at 24-30 hours

The "light" requires 48-72 hours for Lac_V .

5/22/50 Strains of "light" on EMS lac acquire "dark" properties: test this!

See over

Hemizygosity tests
Segregation H229.

4/4/50.

1 Mal+ obtained from D(Mal)

A. Streak on EMS Mal (purify); EMB Mal and Lac
mostly Mal+ Lacv.

Verify from single EMS Mal+ colonies. ✓ verified Mal+ Lacv
~~from 4 EMS cols.~~

B. Streak out on EMB Xyl Test pure Xyl- factor.

31 tests. 2 indicated lac+. Restrict on lac, Xyl.: Both lacv Xyl+

A. 1 additional Mal+. Purify on EMS Mal. check purified colony:
 again: Lacv Mal+ mottled on Mal EMB, but no
 Mal- colonies or sectors.